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Hepatoblastoma Concerns and Growth Hormone Therapy in Small for Gestational Age Children

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INTRODUCTION

Approximately 5% of children are born small for gestational age (SGA).[1] Most of the SGA children present catch-up growth during their first year with completion of the growth recovery by two years of age.[2] After the initial catch-up, most of the height gain is maintained up to adult height. However, children born SGA usually are shorter during childhood and attain adult heights that on average are approximately 1 SD lower than the mean.[2] Approximately 10% of SGA infants do not experience spontaneous catch-up growth and remain short throughout childhood and adolescence and into adulthood.[1,2] These short adults born SGA comprise up to 20% of the total population of short-statured adults.[3]

Growth hormone (GH) therapy for short children born SGA has been explored for nearly 40 years. Many international studies have shown that most of these children benefit from GH therapy by normalizing height during childhood, maintaining a normal growth velocity during the prepubertal years and through puberty, and attaining an improved adult height. In 2001 GH was approved, by the US Food and Drug Administration (FDA) and in 2003 by the European Agency for Evaluation of Medicinal Products (EMEA), for the treatment of short children born SGA who fail to manifest catch-up growth with a height <–2.0 SD by 2 years (FDA) or <–2.5 SD by 4 years (EMEA).[2] In 2008, GH treatment was also approved in Japan for short SGA children who fail to manifest catch-up growth with a height <–2.5 SD

From The Editor's Desk

The US economy continues to struggle and with it the support for independent educational journals such as GGH. Although this journal has not been published quarterly on a regular basis due to lack of financial support, our readership accessed the GGH website with impressive regularity. Thus, an average of 636 distinct viewers accessed articles from the journal every single day this year. This journal's website serves the needs of those who use it as a resource for their educational activities and reference sources. This continued interest of the subscribers to the journal motivated our editorial board to go forward with the publication of this issue.

A new format was instituted; the editorial board prepared each of their articles from high impact papers published in the literature since last year. Additionally I have extracted papers presented at the ENDO 2010 (The Endocrine Society annual meeting, San Diego, California, USA, June 19-22, 2010) considered to be of interest to GGH readers and not otherwise presented/discussed in other pediatric venues. Also I have added pertinent comments to all the articles.

The publication of this issue marks the 26th anniversary since the inception of GGH; a word of thanks to the editorial board for their voluntary participation. Also, an acknowledgment of the 11,000 subscribers of GGH is in order. Your interest in the journal makes our efforts worthwhile and has kept us going in the hope that when the economic slump can be cured we will be able to elicit appropriate financial support to bring to the readership continuous, unbiased, and regular issues of the journal.

Sincerely,
Fima Lifshitz, MD

by 3 years. The FDA, EMEA and Japan approved GH doses for SGA treatment (70/μg/kg/day, 35/μg/kg/day and 33–67/μg/kg/day, respectively) are high because of the presumed GH resistance contributing to the lack of catch-up growth in the SGA population and the results of heightened efficacy at high doses.[2] These doses are up to three times greater than the standard replacement doses used to treat children with GH deficiency; furthermore, higher doses are used in children with marked growth retardation.

GH Therapy for Short SGA Children

The goal of GH therapy in short SGA children is to normalize adult height. To evaluate the impact of GH therapy on adult height in short SGA children, a meta-analysis of randomized controlled trials (RCTs) was performed by Maiorana and Cianfarani.[4] A systematic review of controlled studies was made using as data source the Cochrane Central Register of Controlled Trials, Medline, and the bibliographic references from all retrieved articles describing RCTs up to November 2008. The adult height of the GH-treated group significantly exceeded controls by 0.9 SD. Mean height gain was 1.5 SDS in GH-treated versus 0.25 SD in untreated SGA subjects. No significant difference in adult height was observed between the two GH dose regimens (33 and 67 μg/kg/day). It was concluded that GH therapy seems to be an effective approach to partially reduce the adult height deficit in short SGA children.

The response to GH therapy is highly variable, and therefore additional studies are needed to identify responders versus non-responders. Maiorana and Cianfarani[4] reported that practitioners and policy makers need to address the clinical importance and value of the height gained, including the impact of the height gained on physical and psychosocial well-being, safety and adverse effects, cost of therapy, and patients' expectations.

Adverse Events in GH treated SGA Children

Simon et al[5] reported that clinical trials and a large post-marketing survey have shown that GH treatment is well tolerated in SGA children. However, two particular issues need to be addressed pertaining to this population of SGA patients: the potential risk of malignancy due to high-dose GH treatment and the effects of GH on glucose metabolism. There is a large body of evidence that suggests that low birth weight (LBW) and very low birth weight (VLBW) are associated with a wide range of metabolic and physiological disorders in later life.[2] It is currently unknown whether GH therapy – with higher doses used for SGA children throughout childhood and adolescence – may be associated with an amplification of risk for metabolic consequences such as glucose metabolism, insulin resistance, metabolic syndrome, coronary heart disease and stroke in adulthood.

GH is a known mitogenic agent and insulin-like growth factor (IGF)-I has antiapoptotic effects; therefore, researchers have expressed concern about the oncogenic potential of GH therapy.[6] It is also known that serum IGF-I levels become high among those receiving the high-dose GH; a dose-dependent increase in the IGF-I level has been observed. High IGF-I levels over a prolonged period of time may increase the risk for malignancies; thus, it is currently recommended that IGF-I levels be monitored closely to maintain them within the normal range during GH treatment in SGA children.[5]

The consensus statement of international societies for the management of children born SGA have not reported that LBW has been shown to be associated with increased risk of cancer in general, with the possible exceptions of testicular, and to a lesser extent renal, cancer.[2] In contrast, there is good evidence that high birth weight is associated with an increased risk of cancer, best documented for breast cancer.[2] To date, no reports (including consensus statements of international societies for management of the child born SGA) have addressed the potential relationship of development of hepatoblastomas (HB) during or after GH therapy. A significantly higher rate of HB has been observed among LBW (<2500 g) and VLBW (<1500 g) children.[7-10]

Hepatoblastoma in Children with LBW

Hepatoblastoma is the most common liver cancer in children, occurring most frequently in premature infants, particularly those with LBW or VLBW, aged less than 5 years, especially less than 3 years.[7-10] In SGA children treated with GH the occurrence of HB was mostly considered coincidental.[11] Because data on VLBW and other childhood cancers are sparse, Spector et al[7] examined the risk of malignancy with VLBW in a large data set. They combined case-control data sets created by linking the cancer and birth registries of California, Minnesota, New York, Texas, and Washington states, which included 17,672 children diagnosed as having cancer at 0 to 14 years of age and 57,966 randomly selected control subjects. They found that most childhood cancers were not associated with LBWs. However, a birth weight of 350–1499 g was associated with a considerably high risk of HB (odds ratio [OR]:17.18 and 95% confidence interval [CI]: 7.46-39.54), relative to a weighing ≥2500 g at birth.[7]

Reynolds et al[8] also reported that using California's statewide registry (the California Cancer Registry), a striking elevated risk of HB was found in children from birth to 4 years of age who were born VLBW (OR: 50.57; 95% CI: 6.59-387.97). An analysis of Japanese cancer registry data from 1969-1994 also revealed an increasing trend in HB incidence among children of VLBW.[9] The relative risk of HB among children with birth weights of <1000 to 2499 g compared with children with birth weight ≥2500 g is listed in the Table.[9]

Table. Relative risk of hepatoblastoma in LBW and VLBW children compared to those >2500 g

Birth weight	<1000 g	1000-1499 g	1500-1999 g	2000-2499 g
Relative risk of HB	15.64	2.53	2.71	1.21
P	<0.001	=0.129	=0.001	=0.381

Spector et al[7] also reported that retinoblastoma and glioma (other than astrocytomas and ependymomas) were possibly associated with VLBW. Additionally, VLBW was associated with more than a twofold increased OR for gliomas (birth weight <1500 g, OR: 2.13 [95% CI: 0.71-6.39]; birth weight 1500-1999 g, OR: 3.58 [95% CI: 1.98-6.47]) and retinoblastomas (birth weight <1500 g, OR: 2.43 [95% CI: 1.00-5.89]). There was a significant OR of 1.42 (95% CI: 1.01-1.99) for intracranial embryonal cell tumors associated with birth weights of 2000-2499 g.[7]

Causes of Hepatoblastoma in LBW Children

The causes of HB development in LBW or VLBW children are not fully understood. Infants born with LBW or VLBW may undergo multiple medical interventions in the NICUs at a time in development when antioxidant capacity is decreased and xenobiotic metabolizing enzyme expression is variable; thus iatrogenic causes of HB, such as prolonged oxygen therapy and furosemide use, are plausible.[7-10] The presence of erythropoietin receptors in HB has been also postulated to potentially contribute to this increased incidence of HB because many premature infants with LBW or VLBW receive erythropoietin during their time in the NICU.[7-10]

Latini et al[12] also proposed that perinatal phthalate exposure may play a role in increasing the risk of HB among children with VLBW. Di(2-ethylhexyl)phthalate (DEHP) is the most commonly used plasticizer in polyvinylchloride (PVC) medical devices. In 2001, the Center for Devices and Radiological Health of the FDA reported that neonates in the NICU constitute a population at a particularly increased risk of toxicity because of multiple medical device-related DEHP exposure. In addition, it is well known that in animal models the liver is the most responsive target of the adverse effects of DEHP and that DEHP is a rodent hepatocarcinogen. As a consequence, prenatal and postnatal exposures to potential carcinogens may have synergistic and cumulative actions in producing adverse neonatal effects, especially for VLBW infants.

The GH-IGF-I axis may also be partially involved in HB development. Gray et al[13] reported that the IGF-I axis plays an important role in many diverse cellular functions including promotion of cell growth and cell survival. The main producer of circulating IGF-I and IGF-II is the liver, and the ability of these peptides to mediate mitogenic, anti-apoptotic and differentiation signals is likely to be primarily via the IGF-I receptor. Using RNAase protein analysis (RPA), Gray et al[13] examined the gene expression for *IGF-1* and *IGF-2*, their receptors (*IGF-IR* and *M6P/IGF-2R*), and two IGF binding proteins ([IGFBP]; *IGFBP-1* and *IGFPB-2*) in a series of HBs with corresponding normal liver from the same individuals. The results showed that the expression of many of the IGF-axis genes altered between tumor and normal, and indicated that the IGF-axis may be involved in HB development. Gray and colleagues concluded that the IGF-axis is affected in HB. While there are no definitive explanations on the role of IGF-axis, these alterations may play in the tumorigenesis process. One potential result of these alterations may be local concentrations of IGFs, in combination with reduced levels of IGFBPs, promote clonal expansion of the tumor cells. Further studies are indicated in order to determine the exact importance of the IGF-axis in HB.

Conclusions

Perinatal medicine has rapidly progressed and its sophisticated services have become standard; the survival of infants with LBW and VLBW has increased in recent decades. Treatment with GH for short children born SGA is also increasing – thereby escalating the risk of developing adverse events during and after GH therapy. Although HB occurs most frequently in infants or very young children before 3 years of age, and the usual start of GH therapy is after 2 years, the occurrence of HB has been mostly considered coincidental. However, an early start of GH therapy in short children born SGA has been encouraged[14]; this may increase the potential risks of developing complications – such as HB – for which these patients may be more susceptible than other types of patients being treated with GH.

The precise diagnosis of malignancies in GH-treated children born SGA has not always been reported; some papers may not classify the malignancies precisely, ie other tumors, and perhaps some patients who developed HB were not necessarily included in those reports. Therefore the prevalence of HB in GH-treated SGA patients is not really known.

Diagnosing HB before clinical signs and symptoms develop is important. HB is usually diagnosed as an asymptomatic abdominal mass. Therefore pediatric endocrinologists who follow short SGA children who are being treated with GH should monitor them carefully and repeatedly. Serum α-fetoprotein measurements and if possible, abdominal sonography, should be performed before and during GH therapy to assess for HB. Although the occurrence of malignancy is currently considered coincidental, the families of these children should be informed of the possible occurrence of HB. Furthermore, IGF-I levels should be monitored closely to maintain them within the normal range during GH treatment in SGA children.

Editor's Comment: *The longest post-marketing GH surveillance study has been ongoing in the US for over 25 years – the Genentech National Cooperative Growth*

Study (NCGS). Recently, Bell et al reported more than 20 years of data covering approximately 55,000 patients treated with GH.[15] This is a very valuable analysis of the experience gathered about the use of this drug; the data are reassuring regarding the safety and efficacy of GH.[16] A review of the data by Roberto Lanes was summarized in this issue of GGH. However, the NCGS, as well as other similar studies performed by other companies (eg, KIGS, Pfizer International Growth Database), are not scientific-controlled studies. These post-marketing reporting groups rely on the voluntary reporting by physicians, thus the potential spectrum of potential adverse events may not be comprehensively assessed. The above paper by Yoshikazu Nishi clearly points out this potential weakness; it alerts us to the risk of hepatoblastomas in LBW children who may be treated with GH. The pediatric endocrine community has not hitherto considered this potential risk.

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Practice Guidelines for Pituitary Incidentalomas

From ENDO 2010 The Endocrine Society Annual Meeting, San Diego, June 19-22, 2010

Clinical practice guidelines were highlighted at the Endocrine Society meeting and new evidence-based recommendations on the evaluation and treatment of pituitary incidentalomas were presented by Pamela Freda (chair of the task force that developed the guidelines). A pituitary incidentaloma is an incidentally discovered pituitary lesion. The true nature of pituitary incidentaloma usually remains unknown as most do not result in surgery. In the limited surgical cases available, pituitary adenomas are the most common etiology.

The guidelines recommend the initial evaluation of a patient with a pituitary incidentaloma to include laboratory screening for hormone hypersecretion in all incidentaloma patients, including those with and without symptoms. The task force debated the pros and cons of detailed versus limited screening for hormone hypersecretion ie, stimulation tests versus insulin-like growth factor (IGF)-I and midnight salivary cortisol levels. There was agreement on screening for prolactin, which should be measured in all incidentalomas.

The practice guideline also recommends initial routine screening for hypopituitarism in patients with macro-incidentalomas, both with and without symptoms, but not in all patients with micro-incidentalomas because the incidence of hypopituitarism is not high in these patients. However, the task force suggested screening might be done for patients with large micro-incidentalomas – in the range of 8 to 9 mm. Follow-up testing of pituitary function for hypopituitarism is indicated for patients with macro-incidentalomas 6 months after initial testing and then yearly. However, routine repeated functional testing is not required for patients with micro-incidentalomas when the patient's clinical picture, history and MRIs do not change over time.

Non-surgical follow-up is recommended with clinical assessments and functional testing for patients who do not meet criteria for surgical removal of the pituitary incidentaloma. As for follow-up imaging of non-surgically treated incidentalomas, the guideline recommends MRI of the pituitary in patients with macro-incidentalomas 6 months after the initial scan, and for patients with micro-incidentalomas, one year after the initial assessment.

Finally, the guidelines recommend that patients with pituitary incidentalomas be referred for surgery: if they

have a visual field deficit or signs of compression by the lesion leading to other visual abnormalities; if the lesion abuts the optic nerve or optic chiasm on MRI; if they have pituitary apoplexy with visual disturbance; or if they are found to have a hypersecreting tumor other than a prolactinoma. Other indications for surgery suggested in the guidelines include clinically significant growth of the incidentaloma, loss of endocrine function and unremitting headache, or a lesion that is close to the optic chiasm when the patient is planning pregnancy.

Pamela Freda, MD, Chair of Task Force, Columbia University College of Physicians and Surgeons, New York, USA

Editor's Comment: *The prevalence of pituitary incidentalomas will likely increase as there is more frequent use of brain scanning for multiple purposes. CT scanners first began to be installed in 1974 in clinical settings. Currently over 6000 scanners are in use in the US. The CT has become a commonly performed procedure. Scanners are found not only in hospital radiology departments, but also in outpatient offices. Usage of CT has increased dramatically over the last two decades.[1] An estimated 72 million scans[2] were performed in the US in 2007 – it is likely a higher number today. In Calgary Canada 12.1% of people who present to the emergency with an urgent complaint received a CT scan, most commonly either of the head or the abdomen. The percentage of patients who received CT scans, however varied markedly (1.8% to 25%), depending on the emergency physician who saw the patient.[3] Thus, the practice guidelines are very timely. In children careful assessment of growth progression and sexual development should be evaluated, both at the time the incidentaloma is detected and closely monitored thereafter. Patients with pituitary incidentalomas may find it difficult to accept a wait and see plan without neurosurgical and ophthalmological input; these guidelines should help assure the patient and family.*

Fima Lifshitz, MD

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Inhibitory Role of IGFBP-3 in the Pathogenesis of Asthma

From ENDO 2010 The Endocrine Society Annual Meeting, San Diego, June 19-22, 2010

Insulin-like growth factor-binding protein (IGFBP)-3 is a multi-functional protein known for modulating the actions of insulin-like growth factors (IGFs) in somatic growth and a variety of human diseases such as cancer. Despite the critical role of the IGF system in the pathophysiology of many diseases, limited information is available for its role in bronchial asthma. IGFBP-3 fragments have been identified in asthmatic airway tissue extracts. Whether there is any association between IGFBP-3 and asthma remains elusive.

The researchers performed in vitro and in vivo studies to show that IGFBP-3 blocks specific physiological consequences of asthma in an IGF-independent manner. They used a mouse asthma model with normal mice as well as IGFBP-3 transgenic mice challenged to ovalbumin (OVA). The results show IGFBP-3 suppressed in bronchial epithelial cells from normal mice after OVA challenge. Restoration of IGFBP-3 either by recombinant IGFBP-3 treatment or adenoviral IGFBP-3 gene transfer effectively reduced all physiological manifestations of asthma examined in vivo (airway hyperresponsiveness [AHR], cellular and pathological change in bronchoalveolar lavage [BAL] fluid and lung tissue, and expression of numerous proinflammatory molecules). Furthermore, IGFBP-3 treatment restored airway functions as demonstrated by the reduction of OVA-induced AHR. These unique IGFBP-3 effects were IGF/IGF-I receptor (IGF-IR) independent since IGFBP-3 mutant devoid of IGF binding affinity (IGFBP-3 GGG) had similar effects. The studies using IGFBP-3 transgenic mice further confirmed the effects of IGFBP-3 by demonstrating significant reduction of infiltration of inflammatory cells, cytokine production and OVA-induced AHR compared to that of normal mice after OVA inhalation.

Further in vitro studies using human bronchial epithelial cells demonstrated that IGFBP-3 blocks TNF-α-induced expression of proinflammatory molecules, attenuates the TNF-α-induced migratory response of eosinophils, and negatively regulates TNF-α-induced expression of the key NF-κB regulatory molecules, IκBα and p65-NF-κB, at the post-translational level. Taken together, these results strongly indicated that IGFBP-3 inhibits airway inflammation and airway hyperresponsiveness via an IGF-independent mechanism that involves cross-talk with NF-κB pathway. IGFBP-3 therefore plays a pivotal role in the pathogenesis of asthma, and thus can serve as a potential therapeutic for prevention/treatment of asthma.

Lee Y, Jogie-Brahim S, Harada A, et al. Chonbuk National University, Jeonju, Republic of Korea; University of Manitoba Winnipeg, Canada; and Virginia Commonwealth University, Richmond, Virginia, USA

Editor's Comment: *This is a new and interesting view into the pathogenesis of this common disease. IGF-I is*

known to be involved in airway remodeling in bronchial epithelial cells; interleukin (IL)-17F is able to induce the expression of IGF-I via the Raf1-MEK1/2-ERK1/2-MSK1/p90RSK-CREB pathway in vitro.[1] Another mechanism of allergic airway remodeling may also be via the secretion of the profibrotic IGFBP-3 from IGF-I-stimulated airway epithelial cells during allergic inflammation.[2] Of interest may be the potential role of the IGF system alterations in allergic disease and asthma in growth retardation. The prevalence of short stature (< 3rd percentile NCHS) among children with respiratory allergy (asthma and/or rhinitis) varies from 2–10%. Hauache et al[3] studied IGF-I, IGFBP-3, and growth hormone (GH) serum levels after stimulation tests in prepubertal allergic boys who had not received steroids. All children were short and had delayed skeletal age in relation to chronological age, but bone age was normal for height. The serum levels of IGF-I, IGFBP-3, and GH after stimulation tests were normal and they concluded that in these children a deficiency of GH did not seem to be responsible for short stature.

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Long-term Growth Hormone Use: Safety Profile and Adverse Events

Roberto Lanes, MD

Bell and colleagues recently reported on the safety profile and adverse events detected with the use of recombinant human growth hormone (rhGH) during 20 years of post-marketing surveillance by the National Cooperative Growth Study (NCGS) of Genentech.[1] Additionally an editorial on the subject by Allen was simultaneously published.[2] These two articles are important and should be carefully studied. They describe and interpret the data on the cumulative enrollment of patients treated with rhGH followed by the NCGS. There were 54,996 patients (65% males, 35% females) treated from December 1985 to January 2006. This included 195,419 patient-years of treatment with Genentech's rhGH products. While the overall safety profile of rhGH continues to be favorable, this analysis highlights new areas of concern, while it tends to discard other safety issues.

There were 1559 serious adverse events, including 174 deaths, most of which were unrelated to rhGH. The most common cause of death in the 19 cases believed to be rhGH related were central nervous system (CNS) tumors, particularly occurring in patients with organic GH deficiency (GHD). There were also 5 deaths due to aortic dissection/rupture in patients with Turner Syndrome (TS) and 2 deaths probably due to respiratory/cardiac problems in patients with Prader Willi Syndrome (PWS). There were 11 events consistent with acute adrenal insufficiency (AI), leading to 4 deaths. Of the 4 fatalities, 3 appeared to be associated with infection as were 5 of the nonfatal cases of serious AI. GH is known to affect the metabolism of glucocorticoids and it has a modulating effect on hepatic 11B-hydroxysteroid dehydrogenase decreasing the conversion of cortisone to cortisol. Therefore, endogenous cortisol secretion may decrease after rhGH is initiated in hypopituitary patients and previously unsuspected central hypoadrenalism may become apparent during rhGH treatment. Patients who are begun on rhGH may need to consider glucocorticoid replacement, particularly during stress with increased doses above physiologic maintenance. Patients with hypopituitarism are at a lifelong risk of developing AI, regardless of rhGH use, and need to be counseled and to receive appropriate medical attention during illness as these patients have an increased risk of sudden death.

In the past, leukemia was believed to be a major safety issue associated with rhGH administration. However, there were very few patients with new-onset leukemia in the series. Thus, the data confirmed other reports that therapy with rhGH does not appear to increase the incidence of this cancer in children who do not have any other risk factors that are known to be associated with leukemia.[3]

However, there was an increased risk of second malignancies detected by the NCGS. The patients who received irradiation were at a higher risk of developing second malignancies. Second tumors were seen in 49 of 2500 patients with a prior history of malignancy (excluding craniopharyngioma), or 4.6 cases per 1000 patient years of rhGH treatment. The most commonly detected secondary neoplasms were CNS tumors followed by osteogenic sarcoma. There were 4 malignancies and one meningioma that developed in 16 patients with retinoblastoma. Although the risk of developing a new tumor is increased in any patient with a prior malignancy, regardless of rhGH treatment, this risk seems to be further increased by rhGH. Thus, patients and families need to be made aware of this risk.

There are theoretical risks that may account for the

increased risk of post-treatment tumor development in patients who have received rhGH. The mitogenic and anti-apoptotic actions of GH and insulin-like growth factor (IGF)-I suggest that high-normal levels of free IGF-I may increase the rate of cancers of the breast and prostrate. IGF-I concentrations in the high-normal range are often detected in children and adolescents treated with rhGH, particularly for non-classical indications in which supra-physiological rhGH doses are often administered. The potential relationship between neoplasms, GH use and increased serum IGF-I levels clearly needs to be considered.

Targeted events reported with an infrequent incidence of <1% included scoliosis and slipped capital femoral epiphysis (SCFE), probably associated with rapid growth. New onset cases of scoliosis were not serious and were detected mainly in patients with TS, known to have an increased incidence, independent of rhGH treatment. SCFE was found in PWS associated with obesity, untreated endocrine conditions (hypothyroidism and GHD), trauma, radiation and growth during puberty.

Also occurring in the population of rhGH treated patients was intracranial hypertension (IH), diabetes mellitus (DM), AI and pancreatitis. IH has been previously documented with rhGH treatment, but its mechanism is not clear. It seems to be more frequent in distinct groups of patients who were at a higher risk for this complication, ie, those with chronic renal insufficiency and TS. These patients are known to have a higher risk of IH independent of rhGH therapy.

The incidence of type 1 DM was not increased with rhGH administration, while type 2 DM and insulin resistance seemed to be associated with rhGH use. These alterations appeared to be transient and reversible when GH was discontinued. Pancreatitis was detected in 3 patients with TS and in 7 other patients treated with rhGH; the mechanism linking pancreatitis to rhGH administration is unknown.

There are potential pitfalls on relying on data obtained from post-marketing surveillance studies. Enrollment of treated patients is incomplete, drug exposure is variable, inconsistent compliance may lead to underreporting of adverse events by physicians, and finally reporting of adverse events in these surveillance studies is limited to the period of rhGH treatment, while detection and reporting of subsequent adverse effects depends on reports to monitoring agencies by the physician.

Editor's Comment: *The post-marketing surveillance study reported by Bell et al[1] and reviewed in this issue of GGH by Roberto Lanes, was established, managed and supported by Genentech Inc, manufactures of the first rhGH that was approved for clinical use by the FDA in 1985. This very large and comprehensive project, carried out under the NCGS, is not the only project of this nature. The Pfizer International Growth Study database (KIGS) has also been ongoing and collected data in over 58,000 patients treated with their rhGH product. The post-marketing efforts of the manufactures of rhGH are very important, but they are not scientifically designed studies. Therefore, these data need to be carefully interpreted. There may also be differences in the results between the two large post-marketing studies that should be considered and evaluated to properly understand the differences. For example the KIGS database did not find that rhGH treatment was associated with an increase in the incidence of malignancies[4]; patients with no medical history of risks known to increase the risk of cancer were not at a higher cancer risk with rhGH treatment. However, how long are they planning to look for secondary malignancies later in life? In this issue of GGH Yoshikazu Nishi also points out the potential weakness of such surveillance studies; he calls our attention to the possibility of hepatoblastomas in low birth weight children treated with rhGh.*

More recently, there have been a number of publications that denote interest of the manufactures of rhGH to address the challenges of adherence to the medication regimen in patients receiving rhGH. Non-compliance with rhGH therapy is high[5] and this must be considered in the interpretation of data regarding growth response and/or adverse events and complications of rhGH. For example, it is known that there is lower concordance of height velocity with the duration of rhGH therapy, choice of delivery device and short prescription durations.[6] Adherence to medication administration has been difficult to assess and often determined indirectly by clinical subjective assessments, although newer electronic devices are being used to improve adherence.[7] It may be expected that with improved adherence to rhGH treatment there may be a better growth response – but there may also be more adverse events.

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OBESITY

Fat Mass and Obesity Associated Gene (*FTO*)

Allen W. Root, MD

Several genome wide association (GWA) studies have linked *FTO* (Fat mass and obesity-associated gene - OMIM 610966, chromosome 16q12.2) to weight and obesity risk in children and adults of diverse ethnic origin.[1] Several single nucleotide polymorphisms (SNPs) in intron 1 of *FTO* predispose to obesity while others seem to protect the carrier from this trait. *FTO* encodes a nuclear non-heme iron- and 2-oxoglutarate-dependent dioxygenase that catalyzes the conversion of 2-oxoglutarate to succinate and the demethylation of 3-methylthymine and 3-methyluracil in DNA and RNA, respectively.[2,3] Oxidative demethylation of alkylated nucleic acids is essential for maintenance of an intact genome. *FTO* is expressed ubiquitously in all fetal and adult tissues – particularly in the hypothalamic arcuate nucleus, pituitary, heart, and liver. The arcuate nucleus is the site of synthesis of proopiomelanocortin (POMC) and its anorexigenic product α-melanocyte stimulating hormone and of orexigenic agouti-related peptide (AGRP) and neuropeptide Y (NPY) – essential components of the appetite regulating system. Within the arcuate nucleus, *Fto* is expressed in Pomc synthesizing as well as other neurons. Arcuate nucleus expression of *Fto* is attenuated by fasting and amplified by feeding – particularly of a high fat diet.[2,4]

Boissel et al have identified a consanguineous Palestinian family in which many third generation members displayed impairment of postnatal growth, developmental delay, and death within the first three years of life.[5] They presented malformations involving the CNS (microcephaly, lissencephaly, brain atrophy, neurosensory deafness), heart (ventricular septal and atrioventricular defects, hypertrophic cardiomyopathy), face (anteverted nostrils, thin vermillion borders, retrognathia, cleft palate) and other regions (short neck, brachydactyly, hypoplasia of toenails, ambiguous genitalia). The investigators linked this malformative syndrome to an autosomal recessive, homozygous, loss-of-function mutation in *FTO*. A homozygous guanine to adenine transition at nucleotide position 947 (c.947G⇒A) resulted in substitution of glutamine for arginine at codon 316 (Arg316Gln = p.R316Q), an absolutely conserved position in related orthologous genes in many species. The p.R316Q substitution significantly impaired the function of the enzyme. In addition, in vitro the rate of proliferation and the life span of cultured skin fibroblasts from one of these patients were significantly reduced indicating that these cells aged quickly.

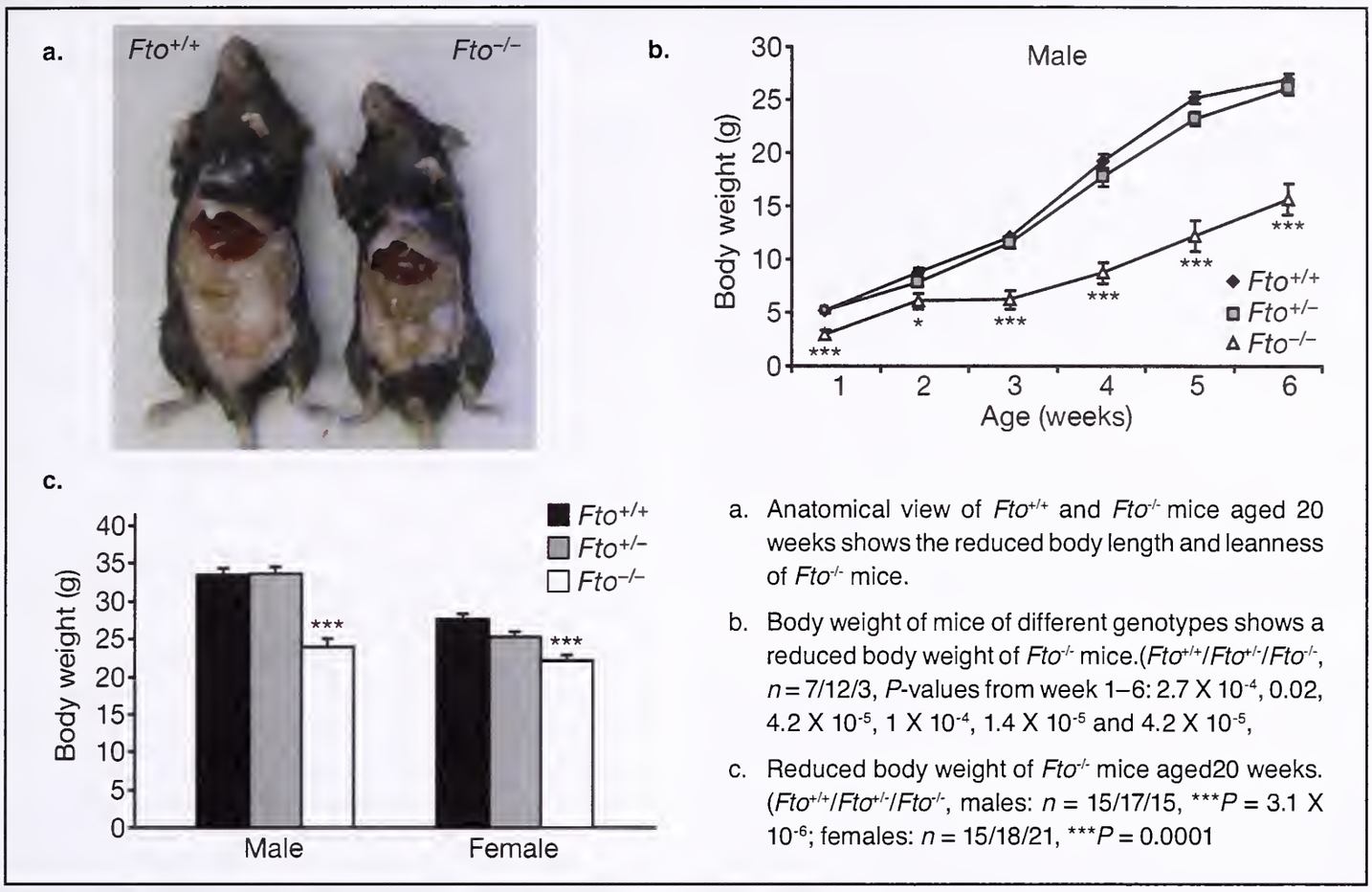
FTO in Experimental Animals

While loss of FTO in humans results in a devastating and lethal complex of anomalies, "knock out" of the murine homolog *Fto* leads to a less severe outcome. Fischer et al developed *Fto*$^{-/-}$ mice by replacing exons 2 and 3 with a neomycin resistant STOP cassette leading to diffuse, germline loss of expression of *Fto*.[6] "Knock out" of *Fto* did not increase fetal wastage; *Fto*$^{-/-}$ fetuses had normal embryogenesis and organogenesis. Although of normal size at birth, weight gain and linear growth of male and female *Fto*$^{-/-}$ neonates faltered within the first week after birth. The growth of heterozygous *Fto*$^{+/-}$ mice was similar to that of wild-type (WT) animals (Figure).

Decreased weight of *Fto*$^{-/-}$ mice was due primarily to lower white fat mass compared to WT animals. Interestingly, brown fat mass was similar in WT and *Fto*$^{-/-}$ mice. White fat accumulates and stores fat and energy, while brown fat metabolizes and expends energy by uncoupling the processes of heat production and ATP generation by generation of uncoupling proteins encoded by *Ucp1*, *Ucp2*, and *Ucp3*. Further studies revealed that the food intake of the WT and *Fto*$^{-/-}$ mice was comparable indicating that relative to body weight the *Fto*$^{-/-}$ animals were actually hyperphagic. The expression of *Pomc* and *Npy* in the arcuate nucleus was similar in *Fto*$^{-/-}$ and WT mice. Energy expenditure in *Fto*$^{-/-}$ mice as assessed by oxygen consumption, carbon dioxide production, and heat generation was significantly greater than in WT mice despite their relative physical inactivity as assessed by determination of spontaneous locomotion. However, increased energy expenditure was not due to greater expression of mitochondrial *Ucp1* in brown adipose tissue or to increased thyroid hormone generation but rather to enhanced sympathetic activity as suggested by higher plasma concentrations of norepinephrine and epinephrine in *Fto*$^{-/-}$ than WT mice. The authors concluded that, in mice, loss of *Fto* increases energy expenditure by enhancing sympathetic activity resulting in futile (ie, non-energy producing) metabolism of triglycerides and fatty acids perhaps in skeletal or cardiac muscle or liver.[7] It is also possible that the expression of *Ucp2* and/or *Ucp3* was increased in brown adipose tissue thus dissipating energy through non-shivering thermogenesis.

Church et al developed a mouse model with a missense mutation (A⇒T) in exon 6 of *Fto* that resulted in replacement of isoleucine by phenylalanine in codon 367 (Ile367Phe = I367F) in the carboxyl terminal region of Fto.[8] This site is not within the catalytic core of Fto but rather in a highly conserved sequence of ~20 amino acids that is required for dimerization of Fto protein and for its optimal catalytic activity. Although Fto^{I367F} localized to the cell nucleus, its expression was reduced and its catalytic activity attenuated but not

Figure. Phenotypic characteristics of Fto-negative mice



a. Anatomical view of *Fto*$^{+/+}$ and *Fto*$^{-/-}$ mice aged 20 weeks shows the reduced body length and leanness of *Fto*$^{-/-}$ mice.

b. Body weight of mice of different genotypes shows a reduced body weight of *Fto*$^{-/-}$ mice.(*Fto*$^{+/+}$/*Fto*$^{+/-}$/*Fto*$^{-/-}$, n = 7/12/3, P-values from week 1–6: 2.7 X 10^{-4}, 0.02, 4.2 X 10^{-5}, 1 X 10^{-4}, 1.4 X 10^{-5} and 4.2 X 10^{-5},

c. Reduced body weight of *Fto*$^{-/-}$ mice aged20 weeks. (*Fto*$^{+/+}$/*Fto*$^{+/-}$/*Fto*$^{-/-}$, males: n = 15/17/15, ***P = 3.1 X 10^{-6}; females: n = 15/18/21, ***P = 0.0001

completely absent. Normal at birth, *both* homozygous Fto^{I367F} and heterozygous Fto$^{I367F/I367}$ male (but not female) mice gained fat mass less rapidly than WT mice after 12 weeks of age; nevertheless, linear growth of mutant mice was comparable to that of WT animals. (The heterozygous Fto$^{I367F/I367}$ mutation may exert a dominant-negative effect on the WT protein.) Relative to WT animals, metabolic rate was higher in both homozygous and heterozygous Fto^{I367F} male mice as estimated by oxygen consumption and carbon dioxide production despite similar levels of physical exertion and brown adipose tissue thermogenic activity. Urinary excretion of catecholamines was greater in mutant than WT animals. In skeletal muscle, expression of the genes encoding the β3-adrenergic receptor, uncoupling protein-2, and catechol-O-methyl transferase was increased. Microarray analyses in white adipose tissue, skeletal muscle , and liver revealed that in Fto^{I367F} mice expression of genes associated with inflammation were decreased and those related to both fatty acid catabolism and synthesis were increased. Hypothalamic expression of *Pomc*, *Agrp*, and *Npy* was not altered in Fto^{I367F} mice. Thus, in a mouse model with less complete loss of Fto activity than in the Fto "knock-out" model, similar manifestations of Fto deficiency were noted but to a lesser extent. Interestingly, the effect of attenuation of Fto activity was not observed in female mice; a somewhat similar observation has been made in humans with a common variant of *FTO*.[9]

The impairment in weight gain and linear growth due to inactivating mutations of *Fto* in mice as demonstrated by Fischer et al[6] and Church et al[8] is primarily due to increased energy expenditure possibly due to augmented adrenergic activity. The more extensive is the loss of Fto function in mice, the more dramatic is the effect. The mechanisms by which FTO regulates energy intake and utilization are unknown. Inasmuch as FTO is a nucleotide demethylase, it is likely that its effects are mediated by differential expression of target genes that are beginning to be identified. Utilizing the male rat as a model, Tung and colleagues[4] stereotactically injected *Fto* cDNA into the arcuate or paraventricular nuclei in order to increase *Fto* expression or shRNA in order to decrease synthesis of endogenous Fto. Increased expression of arcuate nucleus *Fto* lowered spontaneous food intake while impaired generation of Fto enhanced caloric ingestion. Overexpression of *Fto* in the paraventricular nucleus also impaired food intake in the rat model. They further demonstrated (as did Church et al[8]) that alteration in Fto expression did not affect arcuate nucleus expression of *Agrp*, *Npy*, and *Pomc* but enhanced *Fto* expression increased that of *Stat3* and lowered that of *Th* (encoding tyrosine hydroxylase).

Tyrosine hydroxylase is necessary for catecholamine synthesis. Decline in adrenergic hormone synthesis might substantially reduce catecholamine mediated-energy expenditure and thus contribute to obesity in subjects carrying the intron 1 polymorphic variant of *FTO* associated with obesity. Church et al extended these studies to identify possible target genes of *FTO* that regulate fatty acid synthesis and degradation and energy metabolism.[8] Future studies will be directed to deciphering the cellular mechanisms by which FTO regulates energy metabolism and body weight.

In humans, the increased adiposity of patients with polymorphic variants in intron 1 of *FTO* associated with obesity has been ascribed to increased appetite (decreased satiety) and caloric intake rather than to reduced energy utilization.[10] The experimental studies demonstrate that polymorphic variants of *FTO* associated with obesity likely reflect increased FTO activity, while those linked to resistance to weight gain probably attenuate *FTO* expression.

Editor's Comment: *The FTO was identified as a new obesity candidate by a GWA study by Frayling et al[11] in 2007. They found a strong association between SNPs (eg, rs9939609) and adiposity in the first intron of FTO. The predisposition to obesity conferred by this gene was not related to the regulation of energy expenditure, but was mainly accounted for the control of intake of food of high caloric density.[12] The FTO gene rs9939609 obesity-risk allele has also been found to be associated with the loss of control over eating.[13] Given the findings of these and other studies of the molecular physiology of weight regulation (some described by Allen Root above), excess food intake (rather than reduced basal energy expenditure) seems to be the major mechanism for obesity in humans. However, reduced energy expenditure in the pathogenesis of obesity should not be underestimated. In an experimental setup we showed that non-human primates (Bonnet Macaque) who spontaneously developed obesity had reduced energy expenditure compared with their non-obese controls.[14]*

GWA studies, in which hundreds of thousands of SNPs are tested for association with a disease in hundreds or thousands of persons, have revolutionized the search for genetic influences on complex traits.[15,16] The importance for medicine of GWAs were highlighted in the paper by Christensen and Murray.[17] In the past 5 years GWA studies have identified SNPs implicating hundreds of robustly replicated loci (ie, specific genomic locations) for common traits. Nearly 600 GWA studies covering 150 distinct diseases and traits have been published, with nearly 800 SNP–trait associations reported as significant. The GWAs reported through March 2010 are available within the full text of the article by Manolio and colleagues.[18] The reader is encouraged to review the paper in relation to the assessment of risk of disease[19] as well as the series of 3 articles by Attia and colleagues regarding the basic concepts of genetic associations.[20-22]

Fima Lifshitz, MD

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The Metabolically Healthy Obese: A Prospective Study on Risk of Development of Cardiovascular Events

From ENDO 2010 The Endocrine Society Annual Meeting, San Diego, June 19-22, 2010

Obesity is a major health problem with its associated elevated risk of cardiovascular disease (CVD). However, some obese subjects do not have concomitant impaired glucose tolerance, hypertension and dyslipidemia. There are no prospective data whether these metabolically healthy obese subjects are protected against CVD. In the ongoing prospective Dutch PREVEND study (n=7356) normal weight (body mass index [BMI] <25 kg/m^2) at baseline was recorded in 43.2% of participants (3612), while 40.9% (3419) were overweight (BMI 25-29.9 kg/m^2) and 15.9% (1325) obese (BMI >30kg/m^2). In the group with normal weight 39.1% were metabolically healthy (defined as no history of CVD, the absence of diabetes [ADA criteria] and hypertension [JNC 7 criteria] and dyslipidemia [LDL cholesterol >3.50 mmol/L or HDL cholesterol <1.03 mmol/L for men and <1.29 mmol/L for women or triglycerides >1.7 mmol/L or the use of lipid lowering drugs.]) In the overweight or obese groups 13.3% and 6.8%, respectively, were metabolically healthy. During a median follow-up of 7.5 years CVD events occurred in 0.6% of participants with metabolically healthy normal weight, in 1.3% of healthy overweight subjects, and in 1.1% of the healthy obese (P=NS). In metabolically unhealthy participants these percentages were 6.3%, 9.4% and 10.6% for subjects with normal weight, overweight and obesity, respectively (Table). In addition, Cox regression analysis revealed that BMI was not associated with an elevated CVD risk (HR 1.09, p=0.473), when corrected for gender, year of birth, previous CVD and metabolic parameters.

Metabolically healthy obesity represents only a small subset of the total obese population. Metabolically healthy obese persons do not have an elevated CVD risk when compared to normal weight or overweight subjects with a similar metabolic profile.

Verburg FAJ, van Beek AP, Sluiter WJ, et al. University of Groningen, Groningen, Netherlands

Editor's Comment: *Improved fitness may be the factor that determines metabolic health, in both normal weight individuals as well as in those with obesity. Exercise capacity is an independent predictor of all-cause mortality. The relationship is inverse and graded, with most survival benefits achieved in those individuals with an exercise capacity >5 METs. Survival improves significantly when unfit individuals became fit.[1] During a 34-year follow-up, leisure-time physical activity in initially healthy middle-aged men had a graded association with reduced mortality that was independent of BMI, CVD risk, and glucose tolerance.[2]*

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Table. Cardiovascular Events in Metabolically Healthy & Unhealthy Normal, Overweight & Obese Subjects

BMI	Baseline	Metabolically Healthy	CVD Events Metabolically Healthy	CVD Events Metabolically Unhealthy
Normal	43.2%	39.10%	0.6%	6.3%
Overweight	40.9%	13.13%	1.3%	9.4%
Obese	15.9%	06.80%	1.1%	10.6%

BMI: Normal = <25 kg/m^2; Overweight = 25-29 kg/m^2; Obese = >30 kg/m^2.

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DIABETES

Maternal Gestational Glucose Concentration Is Associated with Offspring Insulin Sensitivity and β-Cell Function in Children Aged 5-10 Years

From ENDO 2010 The Endocrine Society Annual Meeting, San Diego, June 19-22, 2010

Evidence suggests that intrauterine exposure to elevated glucose concentrations may be a mediating factor in prenatal programming of offspring disease risk. However, studies examining the effects of maternal glucose concentration on robust measures of insulin sensitivity and β-cell response in prepubertal children are limited. Therefore, the objective of this study was to determine the associations of maternal glucose concentration with robust and physiologic measures of insulin sensitivity and β-cell response. Participants were 21 children aged 5-10 years. Children's insulin sensitivity index (SI) and measures of basal, static, dynamic, and total β-cell response were determined by mathematical modeling using insulin, glucose, and c-peptide values following a liquid meal tolerance test. Dual-energy X-ray absorptiometry (DEXA) was used for the determination of children's percent total body fat (%BF).

Maternal glucose concentration was determined following a 50-gram, 1-hour oral glucose challenge test at 24-28 weeks of gestation and ranged from 75-229

mg/dL. Independent associations of maternal glucose with SI and β-cell response indices were determined by multiple linear regression analyses. Maternal glucose concentration was significantly, inversely associated with SI, independent of %BF (Parameter Estimate ± SE: −0.88+0.27, P<0.01). A significant, positive association was observed for maternal glucose concentration with static β-cell response, independent of %BF and SI (Parameter Estimate ± SE: 1.12+0.41, P<0.05). Maternal glucose concentration significantly impacted insulin sensitivity and β-cell response, independent of adiposity, in offspring at 5-10 years of age. These results suggest that fetal programming occurs both at the pancreas and at the level of insulin target tissues such as skeletal muscle and liver.

Bush NC, Chandler-Laney PC, Granger WM, Rouse DJ, Gower BA. University of Alabama at Birmingham, Birmingham, Alabama, USA and Brown University, Providence, Rhode Island, USA

Editor's Comment: *Maternal glucose concentration in pregnancy appears to be a strong epigenetic factor for fetal programming which impacts insulin sensitivity in offspring during childhood. Perhaps this effect may be imprinted for life.[1] There is growing evidence that even mild gestational diabetes mellitus (GDM) significantly increases the risk of a number of short- and long-term adverse consequences[2] for the fetus and mother, including a predisposition to the development of metabolic syndrome and type 2 diabetes. Maternal and childhood obesity, as well as cardiovascular disease, are also potential long-term consequences of GDM. On the other hand, there is a growing body of evidence suggesting that the risk of many of these consequences can be significantly reduced or eliminated by aggressive treatment of all types of diabetes – including mild GDM.[3] However, there remains, a great deal of controversy over when to begin screening for hyperglycemia in pregnancy and at what level of hyperglycemia aggressive intervention should be initiated.[4-5]*

Fima Lifshitz, MD

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Erythropoietin Provides Diabetes Protection through Direct Effects on Pancreatic β Cells

From ENDO 2010 The Endocrine Society Annual Meeting, San Diego, June 19-22, 2010

Diabetes mellitus is a chronic disorder of insulin insufficiency, resulting in poor glycemic control and vascular complications. The feature common to all forms of diabetes is the insufficient functional pancreatic β-cell mass that is required to maintain euglycemia. Emerging evidence has suggested that erythropoietin (EPO) may exert cytoprotective effects on non-erythroid cells. Interestingly, the EPO receptor (EPO-R) has been found on the pancreatic β cells; however, the biological effects of EPO on the β cells are not well understood.

The effect of recombinant human erythropoietin (rHuEPO) administration was assessed on models of type 1 and type 2 diabetes, using multiple low doses (MLDS) of streptozotocin (STZ) and db/db mice. Mice were given i.p. injections of rHuEPO (50μg/kg) or saline 3 times per week for 4 weeks. In both diabetes models, it was observed that the rHuEPO-treated mice had reduced blood glucose levels compared to controls. The improved glycemic control in the rHuEPO-treated groups was not due to enhanced peripheral insulin sensitivity, but rather enhanced β-cell mass, which was attributed to increased islet proliferation and decreased apoptosis. Treatment with rHuEPO also resulted in enhanced islet angiogenesis. Western blots of isolated islets from rHuEPO-treated C57BL/6 mice demonstrated activation of the JAK2/STAT5 pathway. Bcl-xL, c-Myc, c-kit, and vegf expression levels were upregulated in the rHuEPO-treated mice. To test for the direct biological effects of EPO on the β cells, β cell-specific EPO-R knockout mice were generated. Treatment with rHuEPO failed to provide diabetes protection in these mutant mice following STZ; this supports the direct role of EPO in pancreatic β cells. To assess for essential downstream signaling, β cell-specific JAK2 knockout mice were also tested. These mice also failed to be protected from STZ-induced diabetes development following rHuEPO treatment. Furthermore, enhancement of β-cell mass and angiogenesis were also abolished in rHuEPO-treated knockout mice. These results show that rHuEPO directly inhibits apoptosis, and enhances proliferation and angiogenesis by activating EPO-R and JAK2 specifically in the β cells.

This study demonstrated that rHuEPO can exert beneficial effects directly on the pancreatic β cells. These results may lead to further elucidation of mechanisms of EPO biology relevant to β cells, which may result in novel therapeutic strategies for diabetes.

Choi D, Schroer SA, Wang L, Wu X, Woo M. University of Toronto, Toronto, Canada; St Michael's Hospital Toronto, Canada

Editor's Comment: *The role of rHuEPO in pancreatic cells may bear potential benefits to diabetic patients. Other investigators also found that rHuEPO had no effect on cell apoptosis but it significantly inhibited apoptosis induced by cytokines. It also had no effect on cell insulin secretion, but significantly improved insulin secretion inhibited by cytokines. From these findings, it was concluded that EPO was expressed in NIT-1 cells and EPO could protect NIT-1 cells from apoptosis induced by cytokines.[1] More research is needed before a therapeutic role is considered.*

Fima Lifshitz, MD

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Prevalence of Vitamin D Deficiency and Association with Glycemic Control in Patients with Type 2 Diabetes Mellitus: A Retrospective Analysis

From ENDO 2010 The Endocrine Society Annual Meeting, San Diego, June 19-22, 2010

Hypovitaminosis D has long been suspected to be a risk factor for glucose intolerance. Several reports have suggested an active role of vitamin D (Vit D) in the functional regulation of pancreatic beta cells. Hypovitaminosis D may be an independent risk factor for type 2 diabetes mellitus (T2DM) and metabolic syndrome. The authors estimated the prevalence of 25 (OH) Vit D deficiency in T2DM and the association of Vit D level with HbA1c. They performed a retrospective continuous chart review of 124 patients with T2DM seen at the endocrine outpatient clinic from 2003 to 2008. The data included: age, race, HbA1c, Vit D, PTH level, family history of T2DM, and calcium intake. Vit D levels were divided into 4 quartiles: normal (Vit D >32 ng/dL), mild deficiency (Vit D >25-32 ng/dL), moderate deficiency (Vit D 14-25 ng/dL), and severe deficiency (Vit D <14 ng/dL). SPSS software was used to apply T-test, ANOVA and Chi-square tests for analysis of data. A total of 113 T2DM patients (91.1%) were found to be Vit D deficient (35.5% severe, 38.7% moderate, 16.9% mild). Serum Vit D level was inversely related to HbA1c (Pearson correlation –0.208, P=0.029). Mean HbA1c was higher in patients with severe Vit D deficiency when compared with patients with normal Vit D (7.1% vs 8.18%, P=0.065). Only 90 of 124 patients had their race documented in the medical record (54 White, 33 Black, 3 Asian). The mean HbA1c was higher in Blacks than in Whites (8.59% vs 7.0%, P <0.05), but the mean Vit D level was lower (15.3% vs 23.4 ng/dL, P <0.05). At the time of presentation 8 of 124 patients (6.4%) were receiving Vit D supplementation (2 with normal Vit D levels, 4 with moderate Vit D deficiency, 2 with severe Vit D deficiency).

The results showed a high prevalence (91.1%) of Vit D deficiency in T2DM. Only 6.4% of patients were taking Vit D supplements when first seen at the endocrine clinic, despite regular primary care visits. The inverse relationship between Vit D level and glycemic control in this sample supports an active role of Vit D in the pathogenesis of T2DM. The finding of lower Vit D and higher HbA1c levels in Black patients underscores the importance of aggressive screening and supplementation in the population. Since a majority of T2DM patients are diagnosed and treated by primary care providers, screening and Vit D supplementation as part of routine primary care may improve health outcomes in this highly prevalent condition.

Kant R, Chandra R, Arzumanyan H, Krug E. Sinai Hospital of Balitmore, Baltimore, Maryland, USA

Editor's Comment: *T2DM is associated with obesity that is often the result of increased energy intake, but not necessarily with an appropriate Vit D intake for the calories consumed. These studies were done in Maryland where sunlight exposure may also be lacking, particularly during the winter months. Vit D is*

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known to regulate the expression of over 200 different genes – including the ones related to apoptosis and immune modulation. There has been an important shift in the views about the actions of Vit D during the past decade. In addition to its well-established role in the regulation of calcium metabolism, Vit D deficiency has been associated with the risk of several extraskeletal diseases. It has been suggested that changes in Vit D intake and sun exposure during the past few decades have contributed to the recent increased prevalence of diabetes, including T1DM, as well as other chronic conditions.[1] Is the higher prevalence of T1DM and T2DM and of Vit D deficiency casually related? Well-designed, randomized, controlled trials are needed to determine whether the observed associations are indeed causal.

Fima Lifshitz, MD

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Diabetes in the Desert: What Do Patients Know about the Heat?

From ENDO 2010 The Endocrine Society Annual Meeting, San Diego, June 19-22, 2010

Living with diabetes in hot climates poses unique care challenges. Increasing awareness about the interaction between heat and diabetes should be a priority as more patients are living in regions with high temperatures. Data are sparse on what diabetes patients understand concerning heat or what precautions they should take under extreme heat conditions. A survey of patients attending a Southwestern US diabetes clinic was conducted to gauge types of personal protective measures taken against the heat, knowledge of safe temperatures and exposure times, comprehension of weather data and sources of weather information. From November 30 to December 31, 2009 data were collected in 169 completed patient questionnaires. The mean patient age was 66 years, diabetes duration 15 years, 52% were men, 85% had type 2 diabetes, 62% were non-Hispanic white, 67% took insulin by injection, and 6% were on insulin pumps. Mean HgA1c was 7.9%, 38% had a hemoglobin A1c value ≥8.0%, and nearly 40% had values ≥8.0% during the hottest summer months (July and August). Patients employed a variety of personal protective measures, and 68% limited heat exposure to less than one hour. While respondents typically took steps to protect their diabetes equipment and medication (eg, carrying items in a cooler), 36% simply left medications or supplies at home. Although 72% of respondents indicated they had received information regarding the effects of heat on insulin, a minority of patients acknowledged having received information about the effect of heat on oral medications (40%), on glucose monitors (41%), and on glucose monitoring strips (38%). There was considerable variability in temperatures at which patients would consider taking protective measures. Even though 82% knew the correct definition of humidity, only 55% knew the definition of the heat index. Overall, television was the primary source for weather information (89%).

Many patients had suboptimal glycemic control that placed them at risk for dehydration during the hottest months; as well, they used a medication (insulin) particularly susceptible to heat damage. Most respondents had awareness as to the importance of heat in relation to their diabetes, although knowledge gaps were evident. Increased public awareness of this important topic is needed, and diabetes education should include information about the heat, where regionally appropriate.

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Editor's Comment: *In a recent paper, Westphal and collegaues[1] reviewed MEDLINE publications from 1966 to 2009 that cross-referenced diabetes mellitus, hot temperature, heat, desert, and insulin. It was found that persons with diabetes might have greater susceptibility to adverse effects from heat (ie, increased number of emergency department visits and hospitalizations, increased occurrence of dehydration and electrolyte abnormalities, and higher death rate) than persons without diabetes. Alterations in glucose homeostasis could also occur, and changes in insulin kinetics and stability were possible. The impact of heat exposure on equipment performance (eg, glucose meters) must be considered. The authors concluded that having diabetes places a person at risk for heat-related health problems. Physicians must be aware of possible complications that diabetic patients may encounter in summer heat to prevent problems. Adolescents with type 1 diabetes mellitus may spend the summer at the beach, and they should be aware of the increased risk, particularly those who are not well controlled. Patient educational materials should be developed relating to self-management skills in the heat, and the topic should be included in standard diabetes education programs when applicable.*

As the climate changes, many more people are being subjected to increasing extremes in weather, thus additional education on the health effects of heat on disease and treatment regimens is important. Reid and colleagues have studied the community determinants of heat vulnerability.[2] Four factors explained over 75% of the potential vulnerability variables: a) social/environmental vulnerability (combined education/poverty/race/green space), b) social isolation, c) air conditioning prevalence, and d) proportion of elderly people, and those with

diabetes. In the US, a higher vulnerability was found in individuals residing in the Northeast and Pacific Coast and the lowest in the Southeast. Urban areas and inner cities showed the highest vulnerability to heat.

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GONADS

SF-1 Mutations Cause Isolated Gonadal Dysgenesis and Insufficiency

Raphaël Rappaport, MD

Introduction

Steroidogenic factor-1 (SF-1; also called Ad4BP, encoded by *NR5A1* gene) was a concept proposed in the early 1990s. It was conceived to be an activator of multiple steps in steroidogenesis (a common protein acting as a regulatory element in the proximal region of the cytochrome P450 steroid hydroxylase genes). The corresponding gene orphan nuclear receptor factor-1 (now termed *NR5A1*) was mapped to the long arm of the chromosome 9 in humans. The expression pattern of this gene plays a central role in regulating the transcription of multiple genes involved in adrenal development, gonadal determination and differentiation, and hypothalamic-pituitary control of reproduction and metabolism.

In 1999 Achermann et al[1] identified the first human SF-1 mutation in a patient with the full phenotype (previously observed in *NR5A1* knock-out mice); the patient had primary adrenal failure and sex reversal with 46,XY gonadal dysgenesis with Mullerian structures present. This patient had a de novo heterozygous loss of function SF-1 mutation that was shown to impair the SF-1 ability to activate the promoters of several target genes. The clinical picture was primary adrenal failure that developed after birth, small intra-abdominal gonads with immature seminiferous tubules accounting for the disorder of sex differentiation (DSD) aspect. A second patient with a similar phenotype was reported shortly thereafter.[2] The parents were first cousins and the patient had a homozygous mutation of SF-1.

The possibility that milder or variant changes in *NR5A1* could be associated with different phenotypes was discussed at length in a paper of Lin and Achermann that focused on testis development.[3] In a recent review, Schimmer and White summarized most data on disease and developmental defects.[4] It is now recognized that changes in *NR5A1* can cause developmental and functional disorders of the gonads in 46,XY and 46,XX individuals, without adrenal insufficiency. This is a new and important consideration in the clinical diagnosis of gonadal dysgenesis. Search for *NR5A1* mutations has become part of the genetic work-up in intersex patients even in the absence of adrenal failure, when other known causes have been ruled out.

NR5A1 Mutations and 46,XY DSD

Heterozygous loss of function mutations in *NR5A1* have been found in children and adults with 46,XY and apparently normal adrenal function. The first case was diagnosed in an adult patient with clitoromegaly and primary amenorrhea. She had an absent uterus and impaired breast development. In two further cases ambiguous genitalia were observed with dysgenetic testes and the presence of a uterus (in one case). More recently within two cohorts of 46,XY DSD, *NR5A1* changes could be identified in approximately 15% of the patients. Interestingly, the external genitalia were female in three cases (uterus present in one case, remnants or absence in the other two cases), and ambiguous in 12 cases, most of them lacking a uterus.[5,6]

Most of the *NR5A1* mutations appear to arise de novo. However, in one-third of the heterozygous patients mutations were inherited from the mother in a sex-limited dominant fashion; the mother carried the heterozygous change without presenting ovarian dysfunction and she passed on the gene to her affected sons. This condition may be falsely diagnosed as partial androgen insensitivity syndrome. This sex-limited dominant inheritance can mimic an X-linked disorder. This mode of inheritance is important for the strategy of molecular investigation in these patients.

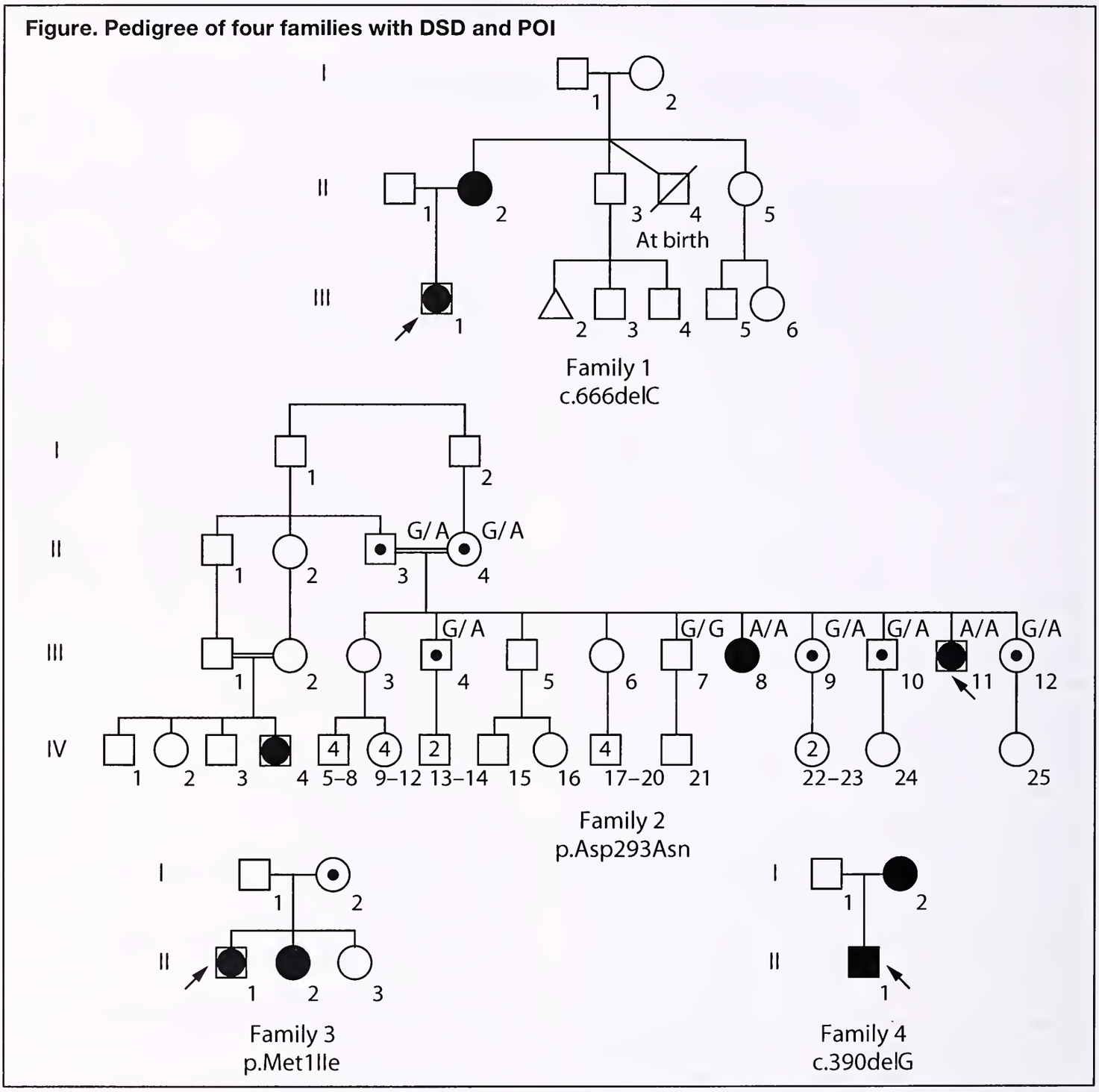
SF-1 (*NR5A1*) Gene Mutation as a Frequent Cause of Primary Amenorrhea in 46,XY Female Adolescents

In a recent paper, Philibert et al[7] turned to a selected population of female adolescents with 46,XY and primary amenorrhea, normal female external genitalia, and clitoromegaly. Subjects were separated into two groups according to their plasma testosterone values. Normal or high values suggested androgen insensitivity or 5-alpha reductase type 2 deficiency. A group of 15 of 31 patients had testosterone levels that

were low for age with elevated gonadotropins. Direct sequencing identified two new *SRY* mutations and one new LH receptor mutation. Five patients had *NR5A1* mutations, two patients had normal external genitalia, and clitoromegaly was present in the other three cases. However, in vitro studies to demonstrate the impact of the mutations were not performed.

It is known that patients with 46,XY DSD include a large phenotypic range, from complete sex reversal (and absence of a uterus) to those much less affected. In an earlier study, the same group reported 24 patients with bilateral anorchidia (vanishing testes syndrome) with or without micropenis.[8] In one patient they found a variant in *NR5A1* reducing to one-half the SF-1 dependant transcriptional activation. Very rarely, a link between changes in *NR5A1* and late and less severe clinical

Figure. Pedigree of four families with DSD and POI



Squares represent male family members and circles represent female family members. Solid squares represent affected 46,XY subjects who were raised as boys, and solid circles represent affected 46,XX subjects. Squares containing solid circles represent affected 46,XY subjects who were raised as girls. Symbols containing a black dot represent apparently unaffected carriers of the mutation. The triangle in Family 1 represents miscarriage, and the symbol with a slash represents a deceased twin. Numbers within symbols indicate multiple siblings. The index patient is indicated with an arrow in each family. Genotyping information is provided for Family 2. The genotypes of the parents of the proband are inferred, whereas all others have been determined by molecular analysis.

changes (such as cryptorchidism and/or, vanishing testis) could be investigated. This should be looked for in at least the familial cases.

SF-1 (*NR5A1*) Gene Mutation and Primary Ovarian Insufficiency in 46,XX Females

Primary ovarian insufficiency (POI) is characterized by primary or secondary amenorrhea, estrogen deficiency and elevated gonadotropins in women younger than 40 years of age. Several genetic causes of syndromic and non-syndromic forms of POI have been identified in recent years. Syndromic forms include monosomy X and the fragile X mental retardation syndrome 1 (*FMR 1* gene). In this group other gene mutations include autosomal recessive mutations in the *APECED*, *EIF2B*, and *GALT* genes. POI can also be associated with the blepharophimosis-ptosis-epicanthus inversus syndrome caused by mutations in the *FOXL2* gene.

A key role for *NR5A1* in ovarian development and function has been observed in mice. It is expressed in multiple cell types in the fetal, postnatal, prepubertal, and mature ovary. There is also evidence of a role at the terminal stages of follicle differentiation and/or ovulation with reduced levels of AMH and aromatase expression in granulosa cells.

Lourenço et al[9] showed, for the first time, that *NR5A1* mutations are associated in 46,XX females with primary ovarian insufficiency and that they may combine with 46,XY DSD in some families, without adrenal insufficiency. They identified new mutations in four families and in two of 25 subjects with sporadic POI. The mode of inheritance of the phenotype in the families is consistent with either autosomal recessive or autosomal dominant transmission. The familial cases are shown in the Figure with associated description in the Table.

NR5A1 (SF-1) mutations may be a significant cause of non-syndromic human ovarian failure. However it remains to be shown if there is a progressive loss of ovarian function in mutation carriers. In addition the incomplete penetrance and variable expressivity as seen in these families may be explained by other endogenous or environmental factors leading to a more complex picture.

Table. Clinical data (from reference 9)

Family 1
II 2 Premature Ovarian Failure, 36yrs, 46,XX POI
III 1 Primary amenorrhea, absence of SSC activity, 17 yrs, 46,XY DSD
Family 2
III 8 Primary amenorrhea, 19 yrs, 46,XX POI
III 11 Primary amenorrhea, signs of virilization, 18 yrs, 46,XY DSD
IV 3 Complete gonadal dysgenesis, 46,XY DSD
Family 3
II 1 Signs of virilization, partial gonadal dysgenesis, 12 yrs, 46,XY DSD
II 2 Secondary amenorrhea, 16 yrs, 46,XX POI
Family 3
II 1 Signs of virilization, partial gonadal dysgenesis, 12 yrs, 46,XY DSD
II 2 Secondary amenorrhea, 16 yrs, 46,XX POI

Editor's Comment: *The reader is referred to a review on SF-1 Mutations in Humans by Tomonobu Hasegawa[10] published in GGH (May 2008 Vol. 24, No. 1) and a minireview on the subject by Schimmer and White.[4] However, the assessment of SF-1 and NR5A1 mutations requires specialized laboratory tests not generally available to endocrinologists who are not practicing in academic medical centers. Furthermore, in the US the medical insurance payer for these patients may not approve the reimbursement for such tests. (In the US, the test is available at Boston University School of Medicine, Center for Human Genetics. They recommend that an insured patient have the tests authorized in advance by the insurance company because payment is quite often denied and patients are left with a bill of $1395 for the SF-1 and NR5A1 gene mutation tests.) However, these tests seem to be necessary for an accurate diagnosis of patients with amenorrhea, signs of virilization, and any other developmental and functional disorders of the gonads in 46,XY and 46,XX individuals.*

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Endocrine Disruptors and Polycystic Ovary Syndrome (PCOS): Elevated Blood Levels of Bisphenol A in PCOS Women

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Bisphenol A (BPA) is used primarily in the synthesis of polycarbonate plastics and is a key monomer in production of epoxy resins. It has been shown that the BPA blood levels are higher in men than in women, a finding that is attributed to androgen and BPA interactions on clearance and sex-hormone binding protein (SHBG) binding properties. Additionally, it has been found that the exposure of experimental animals to BPA adversely influences oocyte development and results in ovarian cystic morphology. The aim of the present study was the determination of BPA levels in women with polycystic ovary syndrome (PCOS) compared to controls, age and body mass index (BMI) matched, as well as the investigation of the association between BPA levels and hormonal and metabolic parameters of studied subjects. Subjects included 100 normal and 71 PCOS women (NIH criteria). Anthropometric, hormonal and metabolic parameters, as well as, BPA blood levels were determined in all subjects. Patients and controls were subdivided and matched respectively in two groups, according to BMI, a lean subgroup and an obese subgroup (Table). Compared to controls, the BPA levels were significantly higher in the lean (1.12±0.10 vs 0.70±0.05, $p< 0.0007$) and obese PCOS women (0.97±0.08 vs 0.74±0.07, $p<0.044$). Additionally, significantly higher insulin and androgen levels were found between PCOS and control subgroups. A significant correlation was found between testosterone ($r=0.188$, $p=0.03$), Δ^4-androstenedione ($r=0.258$, $p=0.003$) and BPA serum levels.

The findings demonstrate that, PCOS women have higher BPA blood levels compared to controls – independent of BMI – and the demonstrated positive correlations between BPA levels and androgens imply that this endocrine disruptor may play a role in the pathophysiology of this syndrome.

Table. BPA Blood Levels in Lean and Obese women with PCOS

Lean PCOS	1.12±0.10*
Lean Control	0.70±0.05
Obese PCOS	0.97±0.08*
Obese Control	0.74±0.07

Data are ng/mL. *P < 0.005 vs controls.

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Editor's Comment: *Human exposure to BPA is nearly universal and recent studies involving this chemical in humans are resulting in growing concerns. Animal studies have documented a variety of endocrine effects of BPA; it acts as an endocrine disruptor. BPA and other endocrine disruptors are finally being considered to play an important role in clinical entities – including PCOS. The association of urinary BPA concentration with medical disorders and laboratory abnormalities was reviewed by Lang et al.[1] Endocrine disruptors have also been shown to alter genital development and puberty, among other clinical conditions. Based on the metabolism of BPA and its endocrine effects, scientists hypothesize that the impact on children will be magnified. Although the Endocrine Society has issued a report expressing serious concerns about endocrine-disrupting compounds, including BPA, the US government health officials still cannot decide whether BPA is safe.[2] The production of plastics[3] will surpass 300 million tons in 2010, therefore we should aim to implement the 5Rs: reduce, reuse, recycle, rethink, and restrain! These actions may benefit all.*

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THYROID

Is Thyroid Hormone Therapy Indicated for Euthyroid Sick Syndrome?

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Greet Van de Berghe (pro side, Catholic University of Leuven, Belgium) and Elaine Kaptein (con side, University of Southern California, Los Angeles, USA) debated the use of thyroid hormone therapy for patients with euthyroid sick syndrome (ESS). The debaters discussed four randomized-controlled trials in ICU patients with prolonged illness treated with thyroid hormone replacement (a total of 190 patients with ESS). Two of the trials used T_3 and two trials used T_4 for the treatment. The T_3 trials showed no change in mortality,

while the T_4 trials showed no change or an increase in mortality. Additionally, in an extensive literature review there were 35 non-randomized controlled studies of thyroid hormone therapy in ESS. For the most part, the results of all 35 trials were inconclusive. All used T_3 and/or T_4 as the active therapy agent. However, the sample size was much too small; for example an ICU study with 23 patients would have needed 142 patients to show statistically significant results. Also the doses of thyroid hormone used in the studies were high, and the wrong hormone may have been utilized. In addition, the effects of malnutrition, medications and other therapies may have played an important role in the outcome of ESS. Sick patients often fast and their nutrition is poor, this depresses serum T_3 levels; proper nutrition quickly corrects the circulating thyroid hormone balance.

It was suggested that a combination of thyrotropin-releasing hormone (TRH) plus growth-hormone releasing peptide (GHRP) may provide benefits in prolonged, critically ill patients. The combination of TRH plus GHRP treatment to correct thyroid hormone levels seemed the most successful in the patients who were receiving adequate nutrition.

There were 14 studies in ESS patients with obesity and calorie restriction, but there were no definitive beneficial effects demonstrated in any of them. Seven other studies of patients with abnormal thyroid findings suggestive of ESS, in various clinical conditions that were treated with thyroid hormone, showed inconsistent results, though one study in patients with coronary artery disease showed decreased systemic vascular resistance. Another study showed an increase in mortality from acute renal failure.

Of the 14 studies performed in postoperative ESS patients who received thyroid hormone therapy, 13 were inconclusive and one showed an increase in cardiac index. A small study in burn patients (14 patients in each group) showed no therapeutic effect, however it would have needed 313 patients in each arm to detect significance.

The evidence in favor of thyroid hormone treatment for ESS is equivocal at best and may increase mortality. Thus, in order to determine the therapy effectiveness and safety in ESS, randomized controlled trials with adequate sample size and appropriate endpoints are needed.

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Editor's Comment: *Euthyroid sick syndrome, also known as low FT_3 syndrome, has a high prevalence in hospitalized patients. In a recent study Iglesias et al described the alterations in thyroid hormone levels in up to 85% of patients.[1] In obese patients, alterations in thyroid hormone levels are often detected. These may reflect ESS related to dietary intake or other factors, not the cause of weight gain or obesity. In premature infants and infants in the NICU, these circulating thyroid hormone alterations are also prevalent; although the debate did not address this issue, it is one of great interest to pediatric endocrinologists. The experimental treatment with TRH and GHRP appeared to improve the circulating thyroid hormones in some patients without other measurable benefits. ESS may be a defense against oxidative stress leading to lower energy expenditures and calorie sparing. This results from a number of homeostatic adaptations in sick patients ie, an increase in glutathione peroxidase, selenium, deiodinase activity type 3, and cytokine interleukin (IL)-6. These lead to decreasing the activation of T_4 and the lowering of T_3 levels. Thus, it may be inappropriate to alter the homeostatic process in sick patients with thyroid hormone treatment. The data suggest that there may be no measurable benefit and there may be increased risks – so, why treat?*

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Increased Miscarriage Rate in Thyroid Antibody-negative Women with TSH Levels between 2.5-5.0 in the First Trimester of Pregnancy

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Studies over the last two decades have demonstrated an increased miscarriage rate in euthyroid women who are thyroid antibody positive. Similarly, women with overt hypothyroidism have an increased rate of spontaneous pregnancy loss. The impact on pregnancy loss with thyroid-stimulating hormone (TSH) levels between 2.5-5.0 in thyroid antibody negative women is unknown. The present abstract is a component of a larger study in southern Italy in which 4562 women were screened for TSH and thyroid peroxidase (TPO) in the first trimester of pregnancy. Women were randomly assigned to a universal screening (US) group or a case finding (CF) group and stratified as high risk or low risk for thyroid disease. All women in the US group and high-risk women in the CF group had TSH and thyroid peroxidase antibody performed immediately. Women in the CF low-risk group had their sera assayed postpartum. Antibody-positive women with a TSH >2.5 were treated with levothyroxine. The results on pregnancy outcome are in press.[1] The present study evaluated the miscarriage rate in thyroid antibody-negative pregnant women with TSH levels between 2.5-5.0 as compared to thyroid antibody-negative women with TSH levels <2.5. None of these women were treated with levothyroxine. In the first

trimester of pregnancy 4123 women were TPO negative with a TSH of ≤5.0 (mean time of screening was 8.8 weeks). The rate of spontaneous pregnancy loss was 6.1% (39/642) in women with a TSH between 2.5-5.0 and 3.6% (127/3481) in women with a TSH <2.5 (p=0.006).

This study demonstrated a significant increase in the rate of spontaneous pregnancy loss in antibody-negative women who have first trimester TSH levels between 2.5-5.0 as compared to antibody-negative women with first trimester TSH <2.5. These data provide further evidence that the normal range for TSH in women in the first trimester of pregnancy is ≤2.5. Future studies are needed to evaluate the impact on the miscarriage rate of levothyroxine treatment in antibody negative women with TSH between 2.5-5.0 in the first trimester of pregnancy.

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Editor's Comment: *These data implied that in pregnancy "compensated hypothyroidism" in thyroid antibody-negative euthyroid women may not be well compensated. Approximately 1-2% of pregnant women receive levothyroxine treatment for overt hypothyroidism. This condition, which commonly has an autoimmune cause, is defined as a low plasma free thyroxine (T_4) concentration and a raised plasma TSH concentration. Another 2.5% of pregnant women have subclinical (compensated) hypothyroidism, which is defined as a raised plasma TSH concentration with a normal free T_4 concentration.[2] It has been suggested that in hypothyroid women anticipating pregnancy (with serum TSH in the lower quartile of normal range) pre-conception adjustment of levothyroxine doses may result in adequate maternal thyroid function.[3] This procedure seems safe and inexpensive; it may be a worthwhile treatment, not only to prevent miscarriage but also in view of the well-known potential effects of even marginal maternal hypothyroid function on the subsequent IQ of the progeny. The data also suggest a role for universal screening in all newly pregnant women with testing for serum TPO antibodies and TSH levels.[4]*

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BONE

Lethal Skeletal Dysplasia Due to Lack of the Golgin GMAP-210

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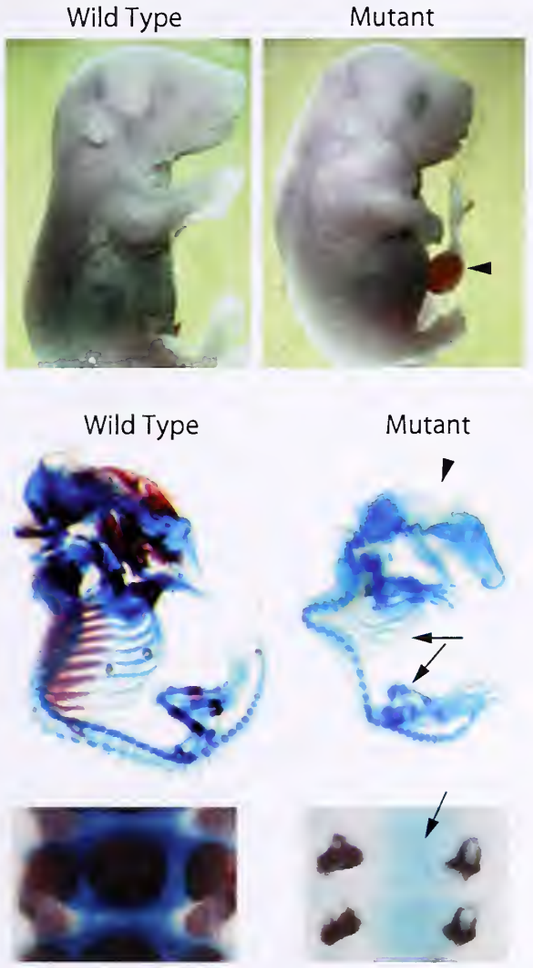
Achondrogenesis is a lethal form of chondrodysplasia. There are 3 types of achondrogenesis - types IA, IB, and II, all of which are phenotypically similar and lethal in utero or in the early postpartum period. Achondrogenesis type IB is due to a biallelic inactivating mutations in *SLC26A2* (OMIM 606718, chromosome 5q32-q33.1) encoding a sulfate transporter. Achondrogenesis type II is due to monoallelic mutations in *COL2A1* (OMIM 120140, chromosome 12q13.1-q13.2) and is characterized by absence of mineralization of the vertebral bodies, sacrum, and pubic bones, a short trunk, and micromelia. In each instance the formation of normal bone is markedly impaired either due to absent synthesis of COL2A1 (the primary collagen of cartilage) or to an abnormality of post-translational modification of cartilage matrix components as the result of structurally and functionally abnormal Golgi apparatus, or disordered sulfation of essential cartilage matrix proteoglycans.

Smits et al[1] have identified the genetic cause of achondrogenesis type IA (OMIM 200600) as biallelic loss-of-function mutations in *TRIP11* (Thyroid hormone receptor interactor 11; OMIM 604505, chromosome 14q31-q32). The protein encoded by *TRIP11* is not only a co-factor for transcriptional signaling by triiodothyronine-thyroid hormone receptor (T3-TR) interaction, but is also essential for structural integrity of the Golgi apparatus. The Golgi apparatus is an intracellular organelle that is indispensable for post-translational modification (glycosylation, phosphorylation, sulfation, proteoglycan formation) of proteins and their sorting, packaging, and directing to sites of action within the cell or for extracellular release. They are comprised of stacks of cisternae into which the basic form of the translated protein enters from the endoplasmic reticulum and is modified as it progresses through the apparatus. The method by which the association between achondrogenesis type IA and inactivating mutations in *TRIP11* was recognized is an example of the experimental induction of random mutations of genes that lead to development of a phenotype of interest in a panel of mice and the subsequent identification of the gene(s) responsible for that phenotype – "forward" mutagenesis.[2] Beier and Herron generated the phenotype by treatment of pregnant mice with N-ethyl-N-nitrosourea (ENU), a teratogen that induces single nucleotide (monogenic) mutations, and then selected for genetic characterization

Figure 1. Effects of *Trip11* mutation in mice



Panel A shows a wild-type fetal mouse and a fetal mouse with an induced nonsense mutation in *Trip11*, the gene encoding GMAP-210, on embryonic day 18.5. The mouse with the mutation has a domed skull, short snout, short trunk, short limbs, and omphalocele (arrowhead).

In Panel B (top), the staining of cartilage with Alcian blue and bone with alizarin red on embryonic day 17.5 in a wildtype mouse and a mouse with the *Trip11* mutation reveals the absence of mineralization in the skull (arrowhead), rib cage, and limbs (arrows) in the mutant. In Panel B, bottom, staining of cartilage and bone in the vertebral columns of newborn wild-type and mutant mice reveals the absence of mineralization in the vertebral body of the mutant (arrow).

the mutated phenotype of interest.[3]

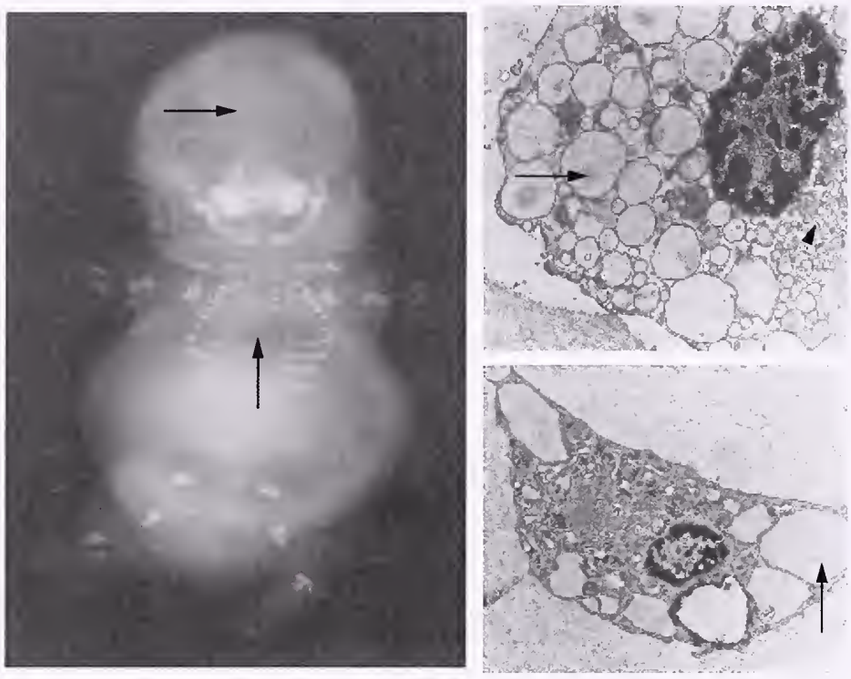
In the Smits et al[1] report, the investigators studied mice with an autosomal recessive phenotype that was lethal in the postpartum period (Figure 1); it was characterized by small thoraces, short limbs and snouts, domed skulls, non-ossified vertebral bodies, delayed mineralization of both endochondral and intramembranous bone, omphalocele, and decreased formation of pulmonary alveoli (likely secondary to impaired thoracic movement). DNA screening revealed a homozygous single nucleotide mutation (c.5003T⇒A) that generated a stop codon (Leu1668X) in *Trip11* and absence of the intact protein product of this gene in the mutagenized mice. Further studies revealed that in the affected mice cartilage formation was markedly askew without columnar formation, marked slowing of progression of proliferating to hypertrophic chondrocytes, impairment of their terminal differentiation, and early apoptosis of chondrocytes. The organization of the Golgi apparatus was disrupted and appeared as a collection of vesicle-like structures rather than an organized cisternal stack. The product of *TRIP11* is also termed Golgi-microtubule-associated protein, 210 KD (GMAP210) and is vital for the structural and functional integrity of the Golgi apparatus. GMAP-210 (and other golgins) direct the fusion of vesicles with Golgi membranes and the transport of selected proteins through the endoplasmic reticulum and Golgi apparatus. In mice with the mutation in *Trip11*, there was impaired glycosylation of proteins and delayed transport of the extremely large heparan sulfate proteoglycan - perlecan - through the endoplasmic reticulum and its intracellular accumulation. Noting that the phenotype of the mutant mice resembled that of neonates with achondrogenesis type IA (Figure 2), the investigators then genotyped *TRIP11* in 10 unrelated patients and identified biallelic frame shift, nonsense, and intronic splice-acceptor mutations in all. The authors concluded that inactivating mutations in *TRIP11* interfered with normal cartilage growth and development by impairing structure and function of the Golgi apparatus of chondrocytes.

It is of interest that TRIP11 also binds to TRβ and enhances T3 dependent transcriptional activity; thus, TRIP11 is a co-activator for T3-TRβ. Assessment of thyroid function in subjects with achondrogenesis type IA or other variants of TRIP11 would be of interest. Inasmuch as thyroid hormone stimulates chondrocyte proliferation and maturation, one might speculate that one mechanism for regulating the effects of T3 on cartilage might be through alteration in intracellular/intranuclear levels of TRIP11.

Editor's Comment: *The osteochondrodysplasias or skeletal dysplasias are a heterogeneous group of over 350 distinct disorders of skeletogenesis. A retrospective analysis evaluated 1500 cases referred to the International Skeletal Dysplasia Registry (ISDR) to determine the relative frequency of specific osteochondrodysplasias and correlation of ultrasound versus radiographic diagnoses for these disorders.[4] Within the retrospective cohort of 1500 cases, 85% of the referred cases represented well-defined skeletal dysplasias, and the other 15% of cases were a mixture of genetic syndromes and probable early-onset intrauterine growth restriction. The three most common prenatal-onset skeletal dysplasias were osteogenesis*

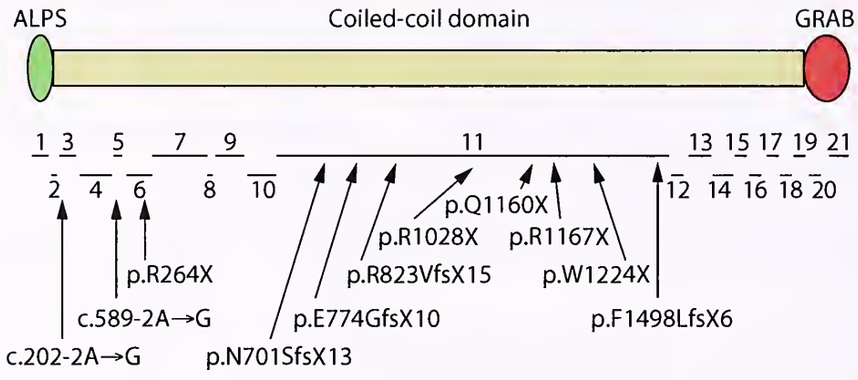
Figure 2. Mutations in *TRIP11* and human achondrogenesis type 1A.

A



Panel A: a radiograph of a 27-week-old human fetus with a diagnosis of achondrogenesis type 1A reveals a lack of mineralization in the skull and the vertebral column (arrows) and short limbs. The electron micrographs at the right show chondrocytes from two unrelated fetuses with achondrogenesis type 1A, with swollen endoplasmic reticulum (arrows) and vesicular Golgi apparatus (arrowhead in upper image).

B



Panel B: the structure of the GMAP-210 protein is shown with the relative location and sizes of its 21 coding exons indicated below. ALPS (amphipathic lipid sensor domain) and GRAB (GRIP-related Arf-binding domain) reportedly mediate interactions between GMAP-210 and membranes in vitro. In the diagram below the protein, arrows point from the mutations that were identified in 10 unrelated patients with achondrogenesis type 1A to their approximate locations within the exons. Two mutations (c.202-2A→G and c.589- 2A→G) affect intronic splice-acceptor sites. Five mutations (p.R264X, p.R1028X, p.Q1160X, p.R1167X, and p.W1224X) are nonsense mutations. The remaining mutations are frameshift mutations. Several patients, including those from consanguineous unions, were homozygous for mutations, whereas others were compound heterozygotes. Some mutations were found in more than one patient.

imperfecta type 2, thanatophoric dysplasia, and achondrogenesis 2, accounting for almost 40% of the cases. The lethal osteochondrodysplasias were rare; their prevalence is estimated at 1:10,000 births. Achondrogenesis type IA (Houston-Harris) is an extremely rare lethal chondrodysplasia with a characteristic severe derangement of endochondral ossification. Molecular analysis in the presented case of achondrogenesis type IA did not reveal mutations in the COL2A1 and SLC26A2 genes, which are known to cause achondrogenesis types IB and type II. The genetic alteration of Achondrogenesis type IA (Houston-Harris) is now elucidated as reviewed by Allen Root in the above paper.

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Lysosomal Pathology and Osteopetrosis

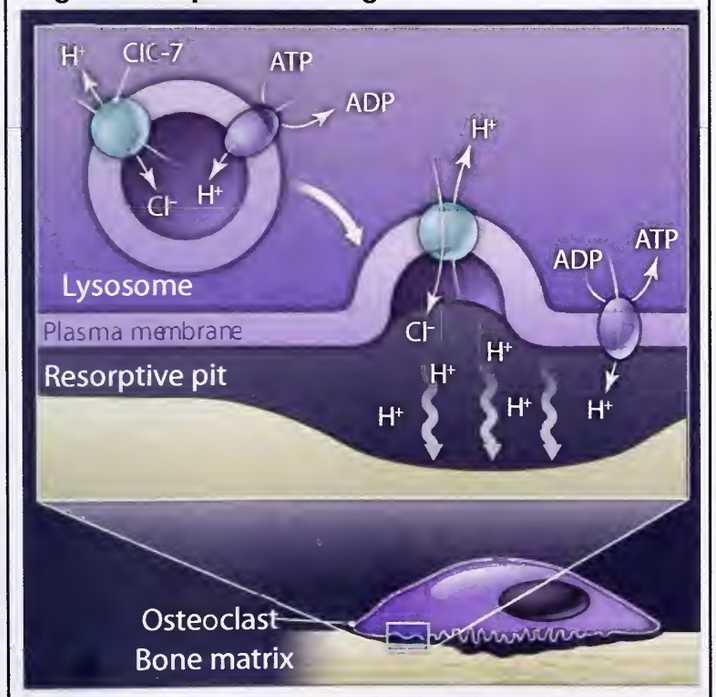
Allen W. Root, MD

Osteopetrosis is a generic term applied to several clinical disorders of varying severity associated with pathologic high bone mass resulting in obliteration of bone marrow which causes pancytopenia and extramedullary

hematopoiesis in the spleen and liver, narrowing of cranial foramina leading to loss of cranial nerve function (sight, hearing), paradoxical osseous fragility, and other manifestations.[1,2] It is due to abnormalities in osteoclast formation or function due to loss of function mutations in at least 10 genes that may be transmitted by autosomal recessive or dominant inheritance patterns. Bone resorption takes place in subosteoclast resorptive pits or lacunae into which are secreted acid (H^+ as hydrochloric acid) that solubilizes the mineral phase of bone and metalloproteases (cathepsin B) that dissolve the protein matrix of bone. The chloride channel in osteoclast lysosomes, which secretes H^+ into the subosteoclast resorptive pit, is encoded by *CLCN7* (OMIM 602727, chromosome 16p13), a protein that is expressed in many tissues including brain (Figure). Inactivating mutations of *CLCN7* result not only in severe to moderate osteopetrosis (depending on the site of the mutation) but also in lysosomal storage, retinal atrophy, and neurodegeneration (OMIM 611490). The CLCN7 lysosomal chloride channel is primarily a chloride-proton (H^+) exchanger—ie, H^+ exits the lysosome through CLCN7 as Cl^- enters and accumulates within this organelle. In the osteoclast's ruffled border, the CLCN7 channel exchanges Cl^- for H^+ in the resorptive pit while a second channel driven by the conversion of ADP to ATP (H^+ - transporting ATPase) secretes H^+ into the resorptive lacuna.[3]

In order to determine whether the H^+ - Cl^- exchange function of CLCN7 was essential or whether CLCN7 might function simply as a passive Cl^- conductor, Weinert and co-workers[4] generated mice in which the H^+ - Cl^- exchange function was abolished leaving the residual protein to function as an uncoupled Cl^- conductor. They did so by mutating glutamate (E) to alanine (A) in codon 245 of *Clcn7*, a site essential for H^+ transport by Clcn7. Mice homozygous for uncoupled *Clcn7* (*Clcn7*$^{unc/unc}$) developed osteopetrosis and associated neural and retinal abnormalities similar to mice with complete loss of *Clcn7* (*Clcn7*$^{-/-}$) but of somewhat less severity. Thus, *Clcn7*$^{unc/unc}$ mice were retarded in growth and died at or before 5 weeks of postpartum age as did *Clcn7*$^{-/-}$ mice. However, compared to *Clcn7*$^{-/-}$ mice there was a more developed ruffled border, the volume of subosteoclast resorptive pits was larger, and bone mass was less in *Clcn7*$^{unc/unc}$ animals. Although initially phenotypically normal, heterozygous mice (*Clcn7*$^{unc/+}$) developed slowly progressive hippocampal neurodegeneration at 5 months of age. The investigators concluded that both the conductance of Cl^- and the exchange of H^+ and Cl^- are essential for normal lysosomal function not only in osteoclasts but in other tissues as well.

Figure. Coupled exchange.



Transporters that import chloride ions in exchange for the export of protons control the function of intracellular vesicles in mammalian cells.

Reprinted with permission Smith AJ, Schwappach B. Science. 2010;328:364-5.

The present data are important because they further unravel the pathophysiology of loss of *CLCN7*. Such data may ultimately permit more physiologically appropriate therapy of neonates and children with inactivating mutations of *CLCN7*. OSTM1 (OMIM 607649, chromosome 6q21) and CLCN7 form a molecular complex that is localized to endosomes, lysosomes, and to the ruffled membrane that caps the subosteoclast resorptive pit, a complex that stabilizes CLCN7. In humans, loss of function mutations in *OSTM1* produce a clinical picture that is similar to that of loss of *CLCN7*.

In man, inactivating mutations of *CLCN5* (OMIM 300008, chromosome Xp11.22) are associated with Dent's disease 1 (OMIM 300009)—X-linked hypercalciuric, hyperphosphaturic nephrolithiasis with microglobulinuria, a phenotype that is mirrored in the *Clcn5*$^{-/-}$ knock-out mouse. In the same issue of *Science*, Novarino and Weinert et al[5] reported the effects of separating renal H^+ - Cl^- exchange from Cl^- conductance by substituting glutamate for alanine in codon 211 (E211A) in *Clcn5* in mice. In *Clcn5*$^{unc/unc}$ mice, clinical and pathophysiological findings were similar to those in *Clcn5*$^{-/-}$ animals indicating the critical importance of H^+ - Cl^- exchange for normal renal tubular function.

Editor's Comment: *Osteopetrosis is a rare human genetic disorder due to markedly decreased bone resorption. In the past, the only gene whose inactivation was known to be responsible for human osteopetrosis[6] was that encoding carbonic anhydrase type II. Now it is known that osteopetrosis may be due to abnormalities in osteoclast formation or function due to loss of function mutations in at least 10 genes that may be transmitted*

by autosomal recessive or dominant inheritance patterns as reviewed by Allen Root above. Sclerosing bone disorders are usually due to mutations in genes required for osteoclast function that can be subdivided according to their clinical presentation, the primarily affected cell type, and the cellular pathways.[7] Clinical aspects of osteopetrosis and the consequences for our understanding of bone biology are discussed by de Vernejoul and Kornak.

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